





Complete mitochondrial genome of the abyssal coral *Abyssoprímnoa gemina* Cairns, 2015 (Octocorallia, Primnoidae) from the Clarion-Clipperton Zone, Pacific Ocean

Romain Gastineau¹, Przemysław Dąbek¹, Kamila Mianowicz²,
Valcana Stoyanova², Artur Krawcewicz², Tomasz Abramowski²

¹ Institute of Marine and Environmental Sciences, University of Szczecin, ul. Mickiewicza 16a, Szczecin, 70-383, Poland

² Interoceanmetal Joint Organization, ul. Cyryla i Metodego 9-9A, Szczecin, 71-541, Poland

Corresponding author: Romain Gastineau (romain.gastineau@usz.edu.pl)



Academic editor: Bert W. Hoeksema

Received: 4 July 2023

Accepted: 9 October 2023

Published: 31 October 2023

ZooBank: <https://zoobank.org/B1AEFD90-0444-4582-9143-A8FF146F5DBA>

Citation: Gastineau R, Dąbek P, Mianowicz K, Stoyanova V, Krawcewicz A, Abramowski T (2023) Complete mitochondrial genome of the abyssal coral *Abyssoprímnoa gemina* Cairns, 2015 (Octocorallia, Primnoidae) from the Clarion-Clipperton Zone, Pacific Ocean. ZooKeys 1183: 81–98. <https://doi.org/10.3897/zookeys.1183.109000>

Copyright: © Romain Gastineau et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Abstract

The Clarion-Clipperton Zone (CCZ) in the tropical East Pacific is a region of interest for deep-sea mining due to its underwater deposits of polymetallic nodules containing economically important metals such as nickel, copper, and cobalt. It is also a region of extensive baseline studies aiming to describe the state of the environment, including the biodiversity of the benthic fauna. An abundant component of the abyssal plain ecosystem consists of sessile fauna which encrusts polymetallic nodules and are vulnerable to potential impacts arising from exploitation activities, particularly removal of substrate. Therefore, this fauna is often considered to have key species whose genetic connectivity should be studied to assess their ecological resilience. One such species is *Abyssoprímnoa gemina* Cairns, 2015, a deep-sea coral from the CCZ whose presence in the Interoceanmetal Joint Organization (IOM) claim area has been confirmed during samplings. In this study, we used next-generation sequencing (NGS) to obtain the 18S nuclear rRNA gene and the complete mitochondrial genome of *A. gemina* from IOM exploration area. The mitogenome is 18,825 bp long and encodes for 14 protein coding genes, 2 rRNAs, and a single tRNA. The two phylogeny reconstructions derived from these data confirm previous studies and display *A. gemina* within a highly supported cluster of seven species whose mitogenomes are all colinear and of comparable size. This study also demonstrates the suitability of NGS for DNA barcoding of the benthic megafauna of the CCZ, which could become part of the IOM protocol for the assessment of population diversity and genetic connectivity in its claim area.

Key words: Deep sea, environmental baseline studies, IOM, ISA, key species

Introduction

The Clarion-Clipperton Zone (CCZ) is a large abyssal area beyond the national jurisdiction in the northern tropical part of the Pacific Ocean, west of Mexico and California and south-east of Hawaii, and governed by the International Seabed Authority (the ISA). This zone has been the area of scientific and industrial interest since the 1950s and is famous for the largest known deposits of

polymetallic nodules—concretions containing various metals, including manganese, iron, copper, nickel, and cobalt (Van Nijen et al. 2018)—covering the seafloor (ISA 2010; Kuhn and Rühlemann 2021). It is also the largest region of exploration activities undertaken by ISA contractors and the scientific community and, as of now, the best recognized region for abyssal plain exploration in terms of environmental conditions (Amon et al. 2022).

Due to the growing interest in exploitation for nodules, an increasing number of sampling campaigns have been taking place in this area, all conducted under the legal framework of UNCLOS, the 1994 Agreement, and a set of Exploration Regulations of the ISA (in place since 2000; ISA 2015). Efforts are underway to develop regulations and establish a legal framework for deep-sea mining to ensure best environmental practices and to prevent, reduce, and control pollution and other hazards, which can potentially interfere with the ecological balance of the marine environment. Research expeditions are ongoing to study the biodiversity and ecological significance of the CCZ to better understand the potential impacts of mining activities and inform environmental management and conservation efforts.

The seafloor is inhabited by endemic species, with an increasing number being discovered and taxonomically described as exploration continues. A large majority of them represent invertebrates such as amphipods, annelids, or ophiuroids (e.g. Amon et al. 2016; Riehl and De Smet 2020; Bonifácio et al. 2021; Bribiesca-Contreras et al. 2021, 2022; Durden et al. 2021; Jones et al. 2021; Laming et al. 2021; Lejzerowicz et al. 2021; Neal et al. 2022; Stewart et al. 2023; Uhlenkott et al. 2023). The CCZ also hosts large benthic foraminifera, including sessile Xenophyophores attached to the nodules (Kamenskaya et al. 2012, 2015, 2017; Gooday et al. 2018, 2020, 2021; Goineau and Gooday 2019; Stachowska-Kamińska et al. 2022; Gooday and Wawrzyniak-Wydrowska 2023).

The CCZ seafloor is also home to an endemic species of deep-water coral, *Abyssoprímnoa gemina* Cairns, 2015 (Cairns 2016), which lives attached to the nodules. The genus *Abyssoprímnoa* is for now known to be monospecific. A major morphological feature of this taxon is the presence of polyps that always occur in pairs, hence its species name *gemina* (Eng. “twins”). Its type locality lies within the German exploration area (BGR claim area), but specimens have also been documented in the neighbouring UK claim area (UKSRL I), all situated in the eastern part of the CCZ.

The eastern part of the CCZ is also where the Interoceanmetal Joint Organization exploration area (IOM claim area) is located (Fig. 1). The IOM claim area is ca 75,000 km², at depths of 2,450–4,750 m, and has been explored by IOM since 1987 (Baláž 2022). Among the various topics of environmental baseline research (e.g. Skowronek et al. 2021; Hikov et al. 2022; Milakovska et al. 2022; Štyriaková et al. 2022), benthic megafauna and nodule-fauna studies are of crucial importance. The main sampling methods applied include photo profiling (video and still imagery documentation) and box-coring of the seafloor. Photo profiling documents the occurrence of benthic organisms living on and just above the seafloor.

Based on this method, the presence of *A. gemina* in the IOM claim area was suggested as early as in 2014 (Figs 2, 3) (IOM Megafauna Atlas, published online in 2021, <https://iom.gov.pl/environmental-research/megafauna-atlas/>) and confirmed later on in the same year by box-coring (Figs 4, 5), before its formal description by Cairns (2016).

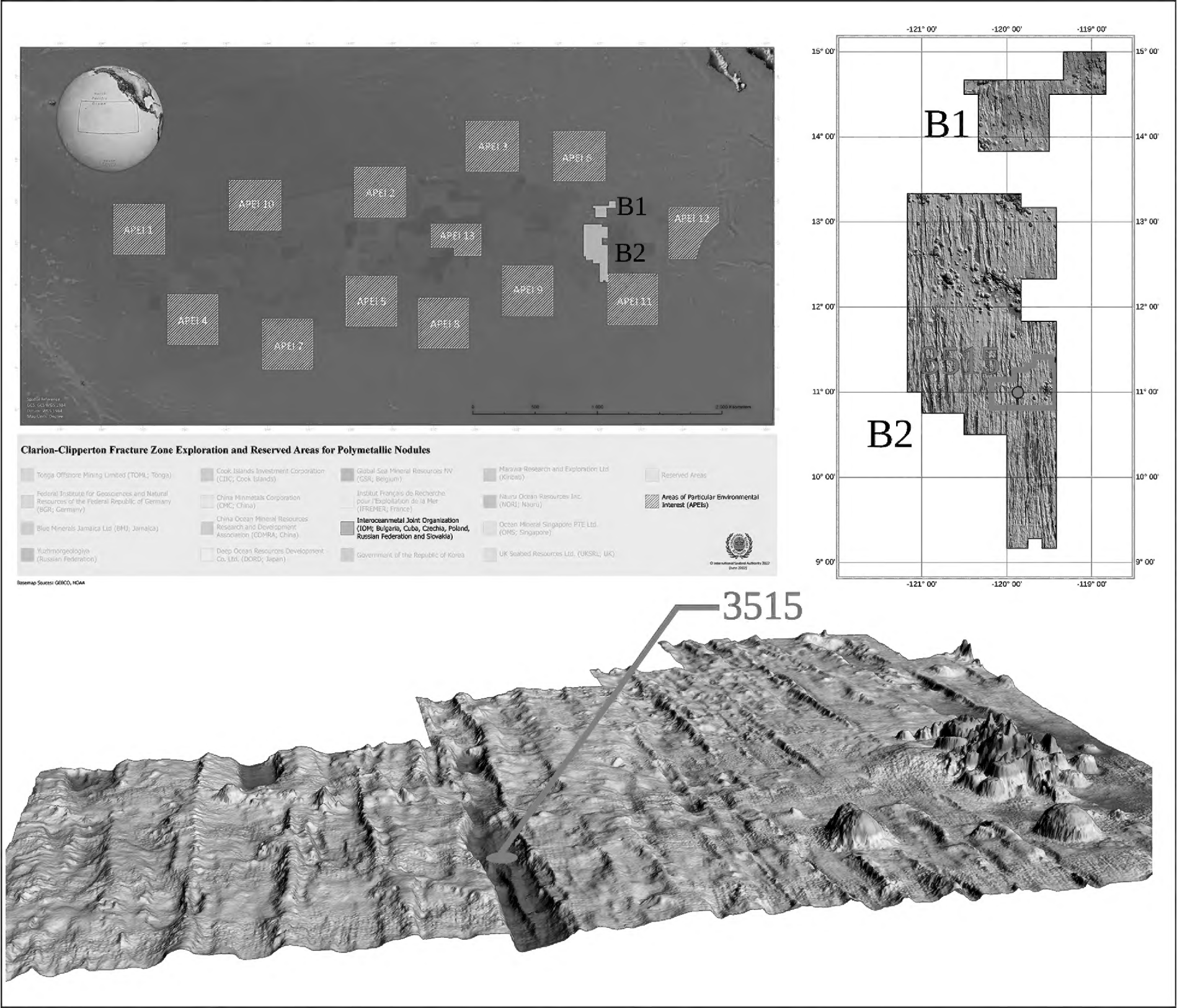


Figure 1. Upper left: location of the IOM claim area in the Clarion-Clipperton Zone, the Pacific Ocean (in orange) (<https://www.isa.org.jm/maps/interoceanmetal-joint-organization/>); upper right: bathymetric map of the IOM claim area B1 and B2, with the location of the exploration block H22 in the B2 claimed area (delimited by red lines) and the location of 3515 sampling station (red dot with the number); bottom: a block diagram of seafloor relief in the exploration block H22 (red dot – location of 3515 sampling station).

During box-corer sampling, undisturbed seafloor sediment, and nodules were retrieved on board of the research vessel and all specimens of benthic mega-, macro-, and nodule fauna were sorted, photographed, registered, and then conserved in ethanol. Attempts were made to taxonomically identify each collected specimen on the basis of its morphology to the lowest level possible. This preliminary identification was planned to be complemented using molecular barcodes (one or more), such as the commonly used genes 18S (of the nuclear small subunit of the ribosomal RNA) or *cox 1* (the mitochondrial cytochrome c oxidase subunit 1 gene).

Although initially not mandatory (ISBA/19/LTC/8), DNA barcoding of specimens from the key taxonomic group (i.e. in the recommendations limited to the representatives of megafauna size class) is currently included in the 2019 “Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in

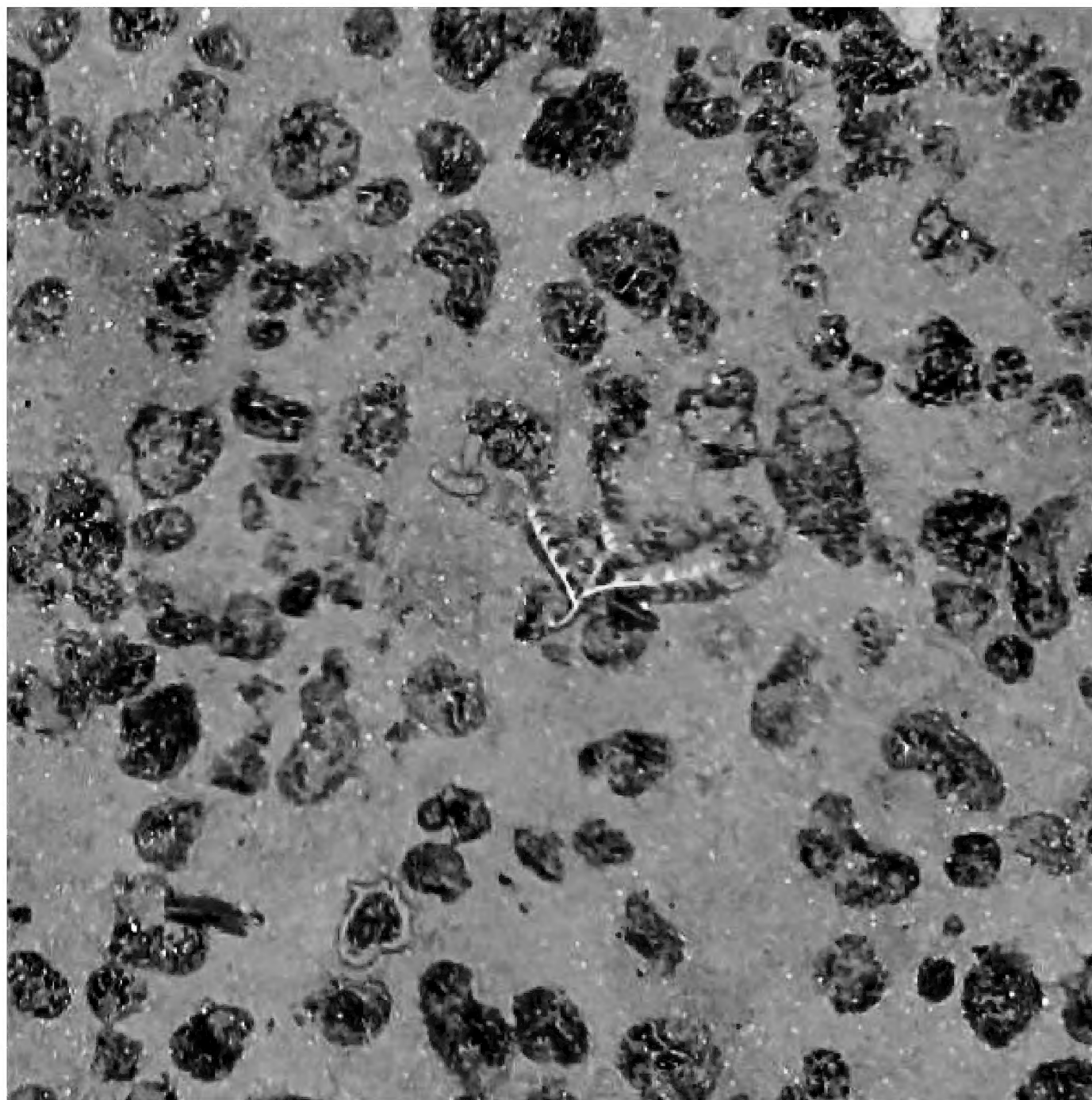
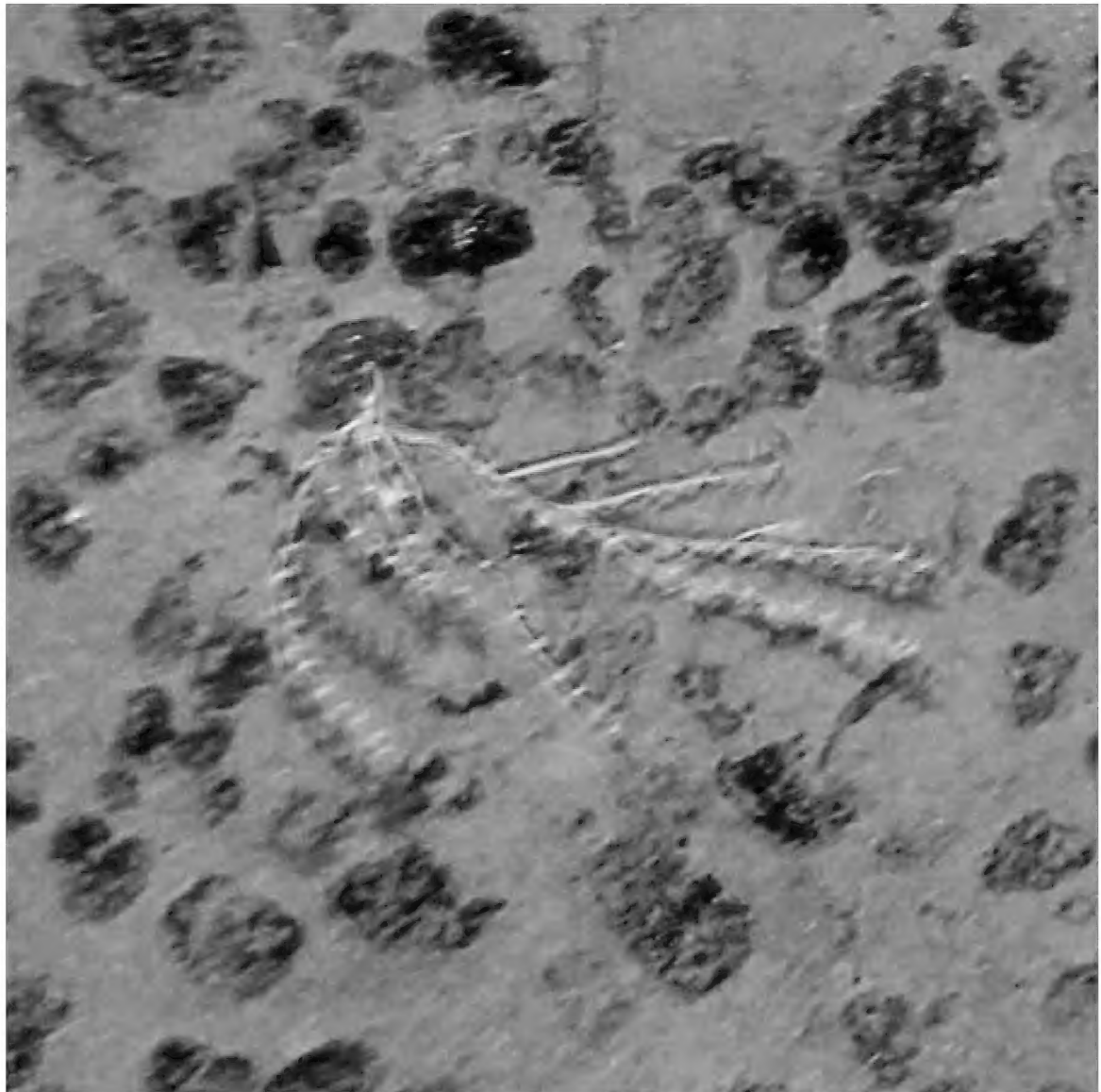


Figure 2. *Abyssoprímnoa gemina* in-situ, as encountered in the IOM exploration zone at ca 4000 m depth (2014).

the Area” (ISBA/25/LTC/6/Rev.2 2022), which are considered obligatory for ISA contractors. Contractors are obliged to “assess regional distribution of species and communities/assemblages as well as genetic connectivity of key and representative species” (section III(b)(15)(d)). Furthermore, it is underlined that: “it is important that molecular studies be undertaken in conjunction with morphological taxonomic analyses” (Annex, 32(a)). However, no specific recommendation is given regarding which molecular marker should be used.

Following these recommendations, IOM recently developed and implemented a DNA-barcoding protocol, which was first applied to the samples obtained during the two exploration campaigns in 2014 and 2019. Principally based on the amplification and sequencing of the two genes mentioned above (*18S* and *cox1*) on all specimens except large Foraminifera, the protocol also tries to use, whenever possible, next-generation sequencing (NGS).

Prior to the current study, there were six sequences of *A. gemina* in GenBank, all of them being partial genes. These sequences originated from two studies. The first one exemplifies the use of DNA barcoding on the CCZ benthic fauna with a focus on Cnidaria (Dahlgren et al. 2016) and is based on two molecular markers, one nuclear (*18S*), and one mitochondrial (*16S*). The second study (Cairns and Wirshing 2018), which was aimed at a large scale multigene phylogeny of Primnoidae, compared with their classification based on morphological characters and relied on four markers, two nuclear (*18S* and *28S*) and two mitochondrial (*cox1* and *mtuS*).



Figures 3. *Abyssoprimnoa gemina* in-situ, as encountered in the IOM exploration zone at ca 4000 m depth (2014).

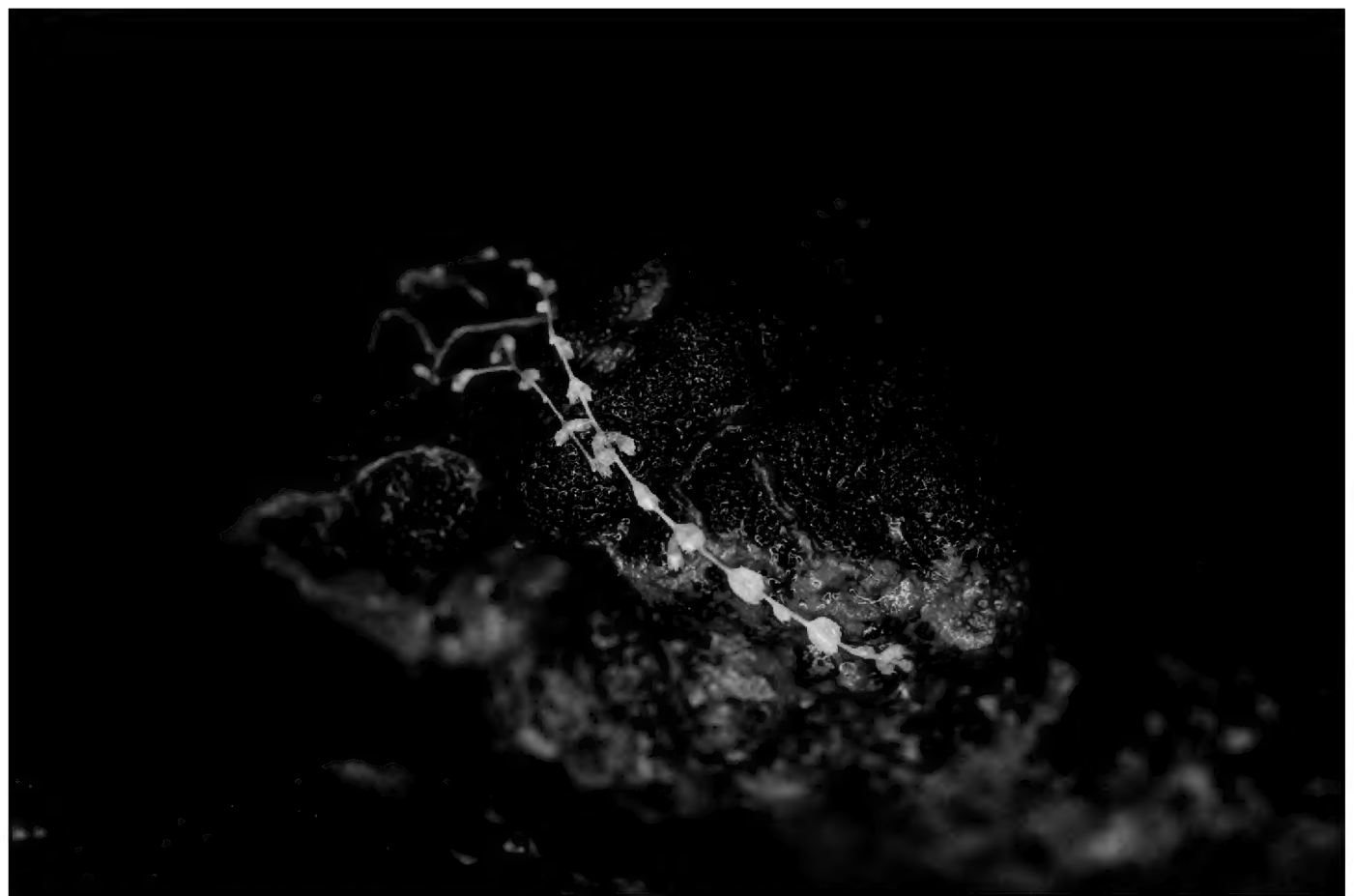


Figure 4. A specimen of *Abyssoprimnoa gemina* collected from the CCZ on a nodule.

A specimen of *A. gemina* was sampled in the IOM area in 2014 and identified under a stereomicroscope a few years later based on the formal description by Cairns (2016). In the frame of the development of a DNA barcoding protocol for the IOM, it was subjected to NGS in 2021. Although it was uncertain if the analysis would be successful due to the time spent by the sample in ethanol, the complete mitogenome and *18S* gene of *A. gemina* were obtained.



Figure 5. Same specimen of *Abyssoprimnoa gemina* with outstretched branches.

The present article describes these findings and their comparison with existing references. The two phylogeny reconstructions derived from the mitogenome confirm the previous placement. The belonging of *A. gemina* to key species is introduced, and the interest of the intergenic regions of the mitogenome for population genetic studies on this species is discussed.

Materials and methods

Exploration and sampling

The specimen used in this study was sampled during the 2014 IOM cruise on 04/24/2014 at station 3515 (−119.7990, −11.1258), at 4241 m depth (Fig. 1). Specimens were acquired by a double shovel box-corer with a surface area of 0.25 m² and penetration depth of 50 cm. The habitat type at the sampling station was described as sediments and polymetallic nodules (according to the ISA habitat type classification, ISBA/21/LTC/15/Corr.1 2021). The specimen was photographed, removed from the nodule it was attached to, moved to a 2.0 mL eppendorf tube with 96% ethanol for storage at 4 °C before being registered under the accession number 2014_28 in the benthic fauna collection of the IOM.

Photography

Photographs of the sea bottom (Figs 2, 3) were taken using a Neptune TS-M1 towed on 8000 m long coaxial wireline. The camera set consists of pressure hull with the CANON EOS 60D camera inside (effective matrix resolution of 18 megapixel). The photo frame (automatic mode) was taken at pre-set time interval (20–30 s) when the current sonar data coincided with a given distance of 4 m above the sea floor, enabling to get photographs of the bottom surface with areal size of about 5 m². Photographs of the specimen brought on board (Figs 4, 5) were taken with a Nikon D700 camera equipped with an AF-S MICRO Nikkor 105mm 1:2.8G ED lens.

Sequencing and bioinformatic analyses

DNA was extracted using a DNeasy Blood & Tissue extraction kit from Qiagen from a 0.8 cm fragment of *A. gemina*. DNA was then sent to the Beijing Genomics Institute (BGI) in Shenzhen (China) where it was sequenced on a DNBSEQ platform. The 60 million 150-bp paired-end reads were assembled using SPAdes v. 3.15.5 (Bankevich et al. 2012) with a k-mer of 125. The mitochondrial genome and the 18S gene were extracted from the contigs file by blastn command line analyses (Camacho et al. 2009). Annotation of the mitogenome was made with the help of MITOS (Bernt et al. 2013) with the genetic code 4 (mold, protozoan, and coelenterate mitochondrial code). The map of the mitogenome was generated using OGDRAW (Lohse et al. 2013).

Phylogeny

Two phylogenetic analyses were conducted based on two recent works on octocorals (Muthye and Lavrov 2021; Muthye et al. 2022). The first one was based on the concatenated sequences of all the mitochondrial protein coding genes (PCG), and the second one on the amino-acid sequence of the DNA repair protein encoded by the *MutS* gene. Datasets from Muthye et al. (2022) were downloaded from OSF and appended with the corresponding sequences of *A. gemina*. All alignments were performed with MAFFT v. 7 (Katoh and Standley 2013) and the -auto option before being trimmed by trimAl v. 1.4.rev15 (Capella-Gutiérrez et al. 2009) and the -automated1 option. For the PCG, the genes were aligned separately, trimmed, and then concatenated using Phyutility v. 2.7.1 (Smith and Dunn 2008). The best model of evolution was chosen with ModelTest-NG (Darriba et al. 2020) on the protein alignment and the concatenated alignment. All maximum-likelihood phylogenies were conducted using IQ-TREE v. 2.2.0 (Minh et al. 2020) with 1000 ultra-fast bootstrap replications in each case.

Data resources

The mitochondrial genome and the 18S gene were submitted to GenBank with accession numbers OR197546 and OR192930, respectively. The raw fasta sequence of the mitogenome and 18S gene, the annotated gbk file for the mitogenome, the alignments used for phylogeny and the complete trees in Newick format were deposited on Zenodo (<https://doi.org/10.5281/zenodo.8100227>).

Results

Sequencing, assembly, and phylogeny reconstructions

It was possible to retrieve the complete mitochondrial genome with redundant endings from the contigs file. However, for unknown reasons, the operon of nuclear rRNA appeared in several pieces that failed to merge. Thus, only the complete 18S gene (1,833 bp) was extracted from the contigs file and later deposited in GenBank (Accession number: OR192930).

The complete mitogenome is 18,825-bp long (GenBank: OR197546). It encodes for 14 protein coding genes, 2 rRNAs, and a single tRNA (Fig. 6), which

is congruent with other octocorals. The best models of evolution returned by ModelTest-NG were TVM+I+G4 for the concatenated PCG and HIVW+I+G4+F for the *MutS* encoded protein. The complete trees, which were too large for publication, are available as indicated in the data resources statement. The figures represent instead highly supported subtrees containing five families. *Abyssoprimnoa gemina* is displayed in both phylogeny reconstructions in a highly supported clade (100%) with all the six other species of the Primnoidae family (Figs 7, 8). It is noteworthy that the mitogenome of *A. gemina* is also colinear with these Primnoidae. There are discrepancies in the rest of the subtrees for what concerns the position of the family Ifalukellidae, represented here by *Trichogorgia capensis* (Hickson, 1904) (Hickson 1904). It is also noteworthy that the support at the nodes for the MutS-inferred tree is in general lower when compared to the multigene tree. It especially concerns two nodes, one associating Primnoidae and Ifalukellidae (66%), and the other associating Chrysogorgiidae to Keratoisididae + Isidoidae.

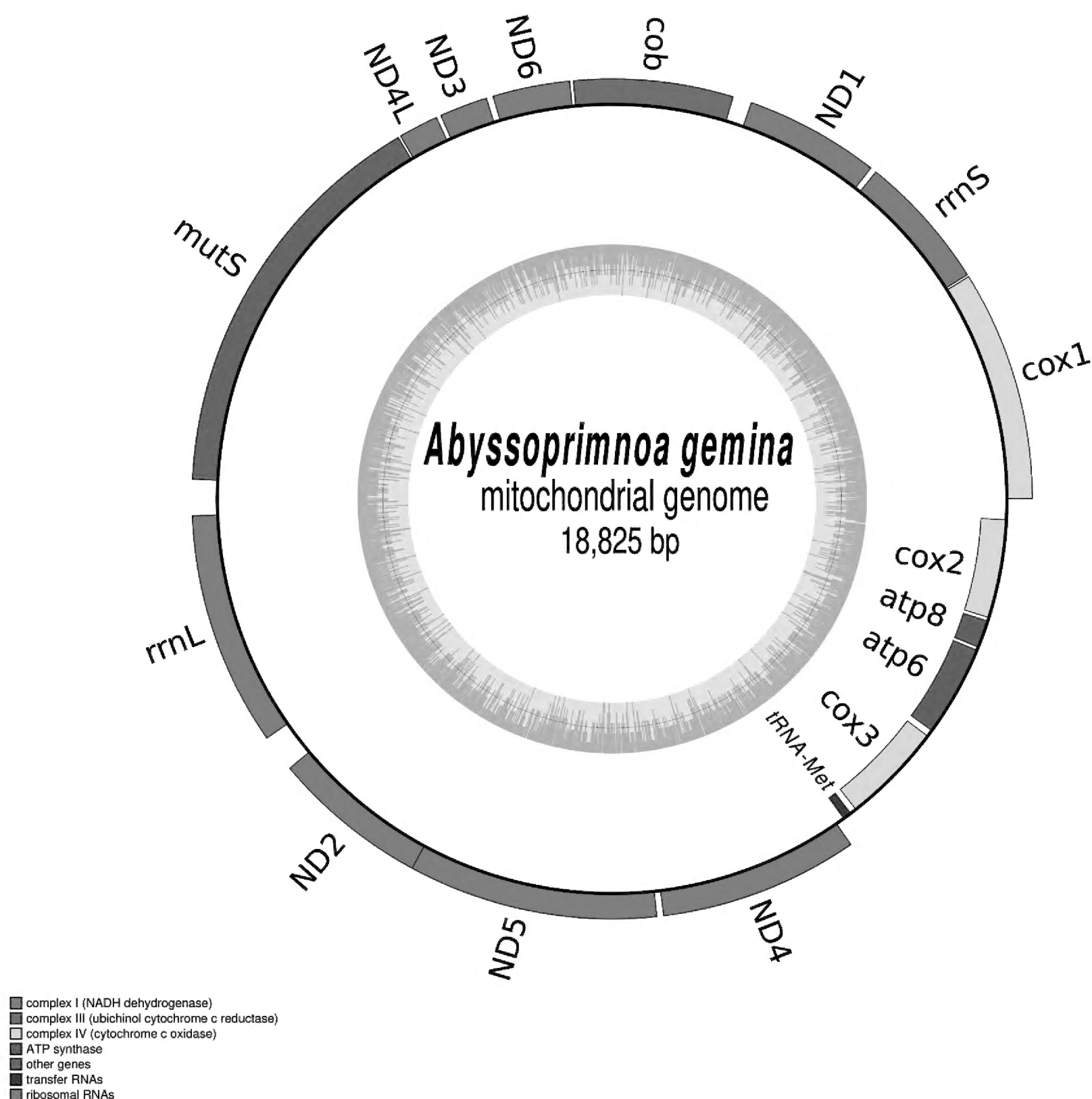


Figure 6. Map of the mitochondrial genome of *Abyssoprimnoa gemina*. The type of genes (e.g. complex I–IV genes, rRNA genes) is represented by different colours whose meaning is explained on the bottom left part. The grey circle represents the GC content.

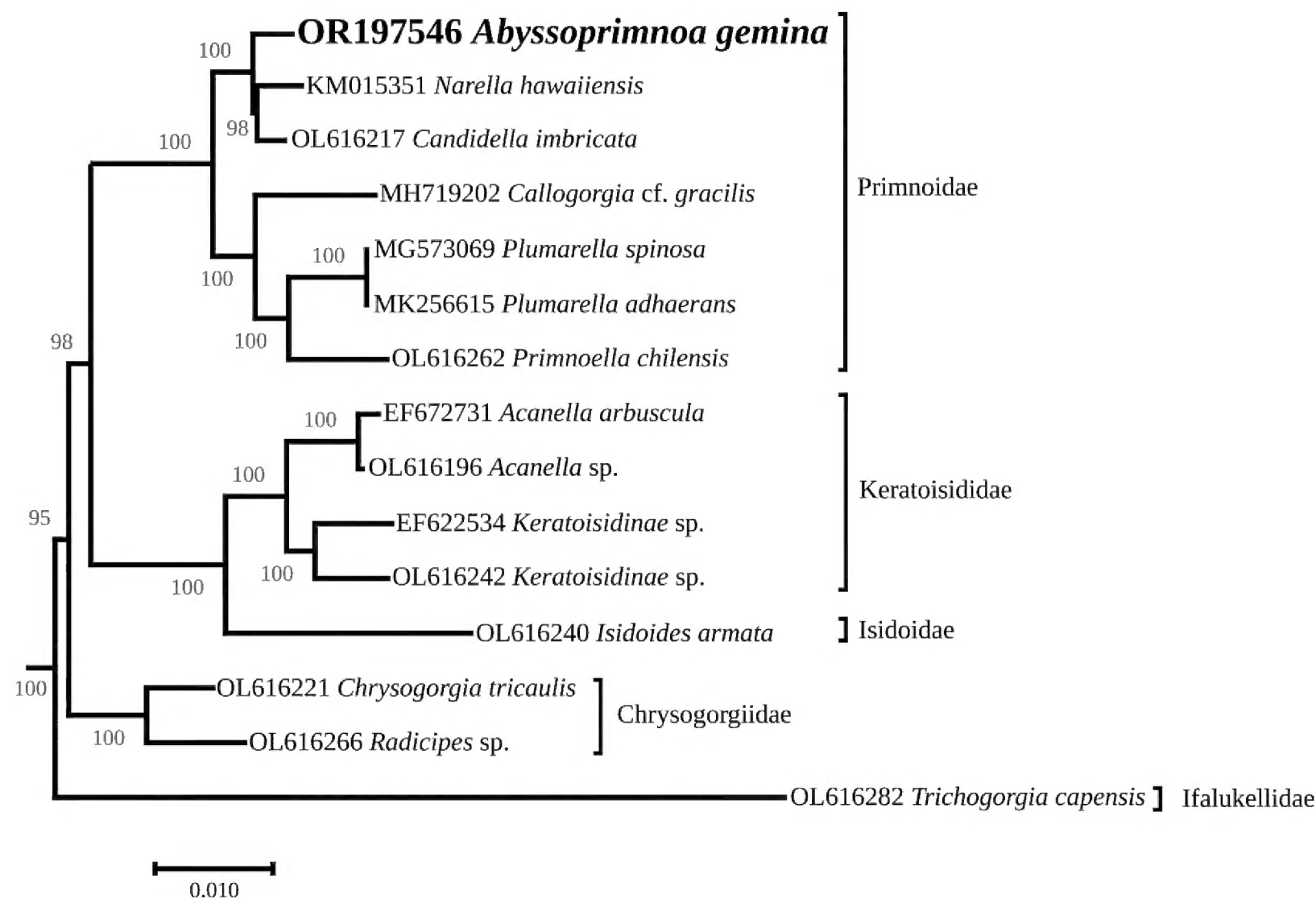


Figure 7. Maximum-likelihood phylogenetic tree obtained from an alignment of concatenated mitochondrial protein-coding genes of 185 taxa of octocorals. The subtree corresponds to a highly supported clade containing among other the family Primnoidae. The name of the families is indicated next to the brackets. The bootstrap values are indicated at the nodes, and the GenBank accession number of the sequence is indicated before the species name. The scale represents the number of substitutions per site.

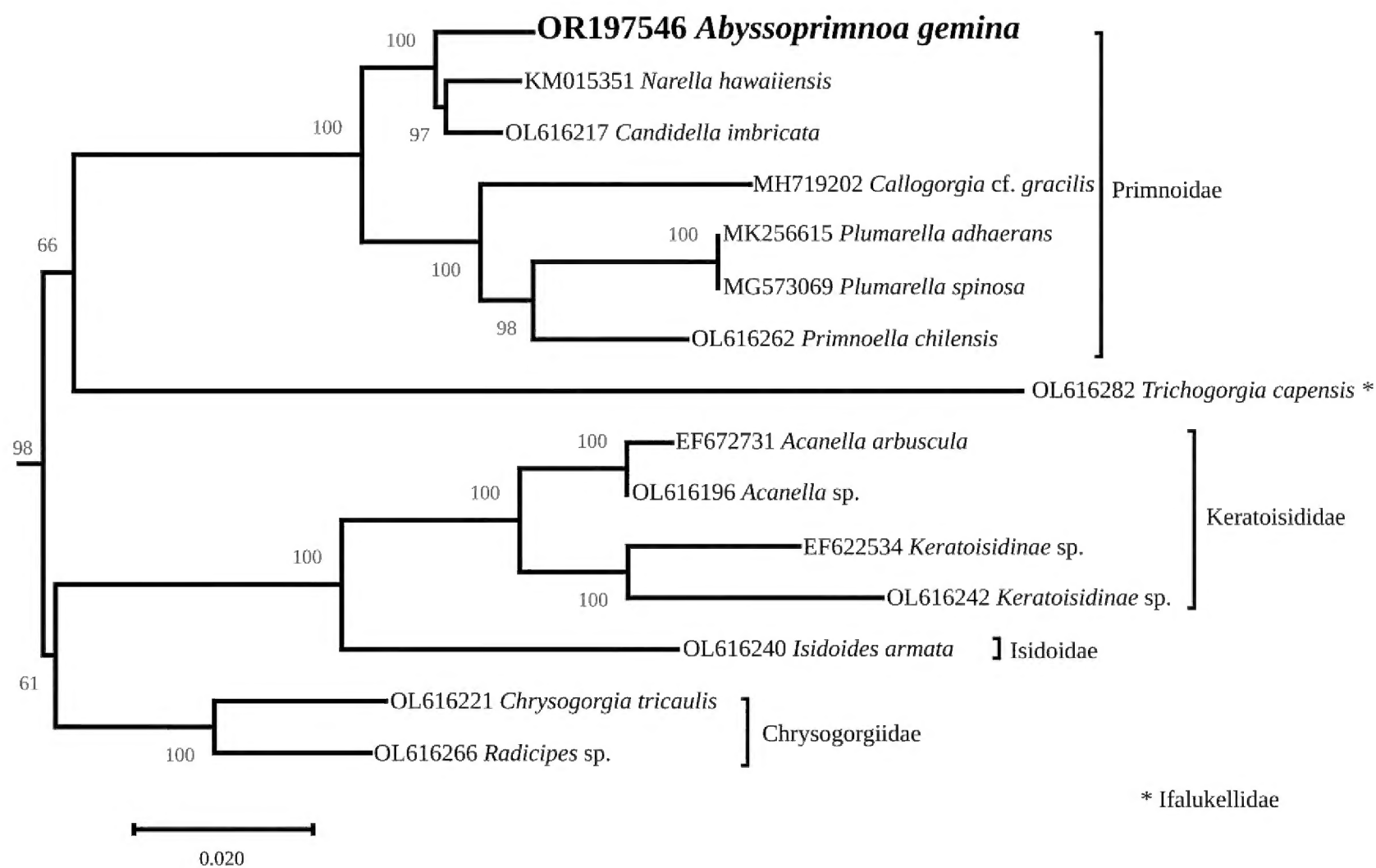


Figure 8. Maximum-likelihood phylogenetic tree obtained from an alignment of 183 *MutS* encoded protein. The subtree corresponds to the highly supported clade containing among other the family Primnoidae. The name of the families is indicated next to the brackets. The bootstrap values are indicated at the nodes, and the GenBank accession number of the sequence is indicated before the species name. The scale represents the number of substitutions per site.

Comparison with available sequences: DNA barcoding

Comparison of the sequences of the mitochondrial PCG with the references from GenBank showed a complete identity. The sequences concerned were the partial *cox1* (MG986971) and *MutS* (MG986922) genes. For what regards the partial mitochondrial 16S gene (KX384626), there were two polymorphisms found at the nine last base pairs of the 3' ending. There was a single indel in the 5' ending of sequence KX384618, differentiating it from the 18S complete gene obtained in the course of this study. Both sequences, however, differed from MG980108 by the presence of a 9-bp deletion at the 3' ending of the sequence.

Discussion

As stated above, the mitogenomes of *A. gemina* and the other six Primnoidae species are colinear, clustering together in both phylogeny reconstructions. The size of the *A. gemina* mitogenome (18,825 bp) is slightly smaller compared to the other ones, which range between 18,838 bp for *Narella hawaiiensis* Cairns & Bayer, 2008 (Cairns and Bayer 2008; Figueroa and Baco 2015) to 19,037 bp for *Plumarella spinosa* Kinoshita, 1907 (Kinoshita 1907; Choi et al. 2020).

To our knowledge, *A. gemina* is so far only the second representative of the CCZ benthic fauna to have its mitochondrial genome sequenced. This can be easily accounted for by the scarcity and small sample sizes, as well as the difficulty to accurately identify them taxonomically. Due to the small size of most benthic macrofauna specimens, obtaining a sufficiently large amount of DNA might result in the destruction of large fragments of their colonies, which may be a problem when depositing voucher specimens. In addition, in the case of the IOM samples, it must be remembered that they had been kept in ethanol for seven years prior to DNA extraction, which could have jeopardized the outcome of the sequencing by lowering the quantity and quality of DNA to be used as a template for the preparation of the library.

During this study, we noticed that an NGS-based approach could be considered when the size of the specimen is large enough and belongs to a known species, even if the biological material is not fresh. In addition to the original aim of performing DNA barcoding on the CCZ samples for comparison with databases, we also obtained the complete mitogenome and obtained phylogeny reconstructions, which provide more evidence for the proximity between *A. gemina* and the genus *Narella* Gray, 1870, as already demonstrated by Cairns and Wirshing (2018).

As mentioned above, *A. gemina* was not the first organism from the CCZ benthos to be studied using NGS, as five years earlier the mitochondrial genome of the demosponge *Plenaster craigi* Lim & Wiklund, 2017 (Lim et al. 2017) had been obtained by Taboada et al. (2018a). Contrary to our study, which is based on a single individual, the subsequent article from Taboada et al. (2018b) was based on 75 Porifera specimens. Their aim was to find nuclear microsatellite loci among the contigs obtained after assembly for studies at the population level, and to test their distribution among other specimens by PCR.

Taboada et al. (2018b) justified their approach on the grounds of the small numbers of polymorphic sites in the mitochondrial genome of Porifera. A similar problem is likely to occur in octocorals, such as with *A. gemina*. The high conservation level of octocoral mitogenomes has already been noted by

several authors (Muthye and Lavrov 2021; Muthye et al. 2022; Ramos et al. 2023), which can be a problem in population genetics. There is an absolute conservation observed among mitochondrial PCG of our sample and others in the databases. Even if polymorphisms were spotted between the rRNA genes, they were always located at the endings of the reference sequences, all obtained by Sanger sequencing. Although not rejecting the possibility that these polymorphisms do exist, more samples need to be sequenced to confirm this.

An additional interest of sequencing a complete mitogenome is that it also includes the non-coding parts. As stated above, the conservation of mitochondrial genes among Anthozoa is reputedly high, leading authors to deem them as less efficient for studies at the population level than the nuclear gene (Shearer et al. 2002). This is exemplified by *Balanophyllia elegans* Verrill, 1864 (Verrill 1864) for which partial *cox1* gene has been found invariable among 18 populations distributed along 3,000 km (Hellberg 2006). However, studies on mitochondrial intergenic regions were used to define haplotypes among Anthozoa (Smith et al. 2004). For example, up to eight haplotypes were found for *Galaxea fascicularis* (Linnaeus, 1767) (Watanabe et al. 2005). Smith et al. (2004) studied a non-coding region between *cox1* and *cox2*, while Watanabe et al. (2005) used the intergenic spacer between *cob* (or *cytb*, coding for cytochrome b) and *ND2* (coding for the second subunit of the NADH dehydrogenase). In *A. gemina*, there is also a 155 bp intergenic spacer between *cox1* and *cox2* (both genes located on different strands), but *cob* and *ND2* are not contiguous in this species. Instead, we found other intergenic sequences of potential interest, for example between *ND1* - *cob* (131 bp) and *rrnL* - *ND2* (265 bp). Although shorter than the regions sequenced by the aforementioned authors, they could still present polymorphisms of interest.

In addition, reads such as those obtained in the course of this study can also be processed through pipelines such as HybPiper (Johnson et al. 2016) supplemented with a proper database (e.g. Quek et al. 2020) to directly extract from them orthologous loci putatively polymorphic. The data gathered can be later employed in phylogenomic studies as recently exemplified by Quek et al. (2023), but they might also be used for studies at the population level.

During the exploration phase of the CCZ, the contractors are obliged to gather environmental data (describing the state of environment) to inform the baseline against which possible environmental impacts arising from exploration for marine minerals will be assessed (ISBA/25/LTC/6/Rev.2). Collecting information on “regional distribution of species and communities/assemblages as well as genetic connectivity of key and representative species” is one of the requirements imposed on the contractors by the regulator; however, there is no recommendation with respect to the criteria for identifying key species. Rabone et al. (2023) proposed a list of key taxonomic groups of macrofauna (tanaids, isopods, and polychaetes), as well as megafauna (asteroids and ophiuroids). The term “key” as used here means most abundant and dominant (“major”) groups of benthic fauna (Simon-Lledó et al. 2020). To our knowledge, there is limited information on connectivity of benthic fauna in the CCZ, whether it refers to key species or not (Taboada et al. 2018b; Kersten et al. 2019; Janssen et al. 2019; Patel et al. 2020; Bribiesca-Contreras et al. 2021). It represents one of the knowledge gaps presently identified (Amon et al. 2022), which must be addressed in order to inform evidence-based environmental management. The point of the current study also belongs to IOM’s strategy to deal with this issue in the eastern part of the CCZ.

If we refer to existing works, *P. craigi*, the aforementioned species of demosponge found on polymetallic nodules, was proposed by Taboada et al. (2018a, 2018b) as a key species and a model organism to study biogeographic patterns at varying spatial scales, with the help of molecular and genomic tools. The three reasons behind this choice (Taboada et al. 2018b) were: (1) a high dependence on polymetallic nodules which provide the substrate for adults, (2) a filter-feeding mechanism of obtaining nutrients from particles suspended in water, and thus high vulnerability to increased turbidity, also resulting from collector plumes (Weaver et al. 2022), and (3) presumed (analogous to other sponges) short pelagic larval durations (lecithotrophic larvae) with relatively limited dispersal ability. Combination of these three traits of *P. craigi* can, in turn, result in high vulnerability of this species to temporal (increased turbidity) and permanent (substrate removal) changes in environmental conditions caused by deep-sea mining.

Abyssoprímnoa gemina shares at least two (or three if lecithotrophic larvae are confirmed) of these traits (in general coral larvae are considered lecithotrophic (Rodd et al. 2022)), and therefore we propose that it can be considered a key species. In general, cold-water corals, including deep-sea corals, are regarded vulnerable to anthropogenic impacts (e.g. Cordes et al. 2016). In addition to the traits already mentioned, these corals are also characterized by slow growth and recovery rates (Roark et al. 2009).

If *A. gemina* is to be later considered a key species, it will imply that further genetic investigations will have to be conducted on it, building on the current work. Depending on the results of the next IOM cruises, perhaps additional specimens of *A. gemina* will become available for sequencing. The other ISA contractors and members of the scientific community working on the CCZ could also participate to improve our knowledge on *A. gemina* from their own exploration area following the protocol described here.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

Conceptualization: RG. Funding acquisition: TA. Investigation: RG, TA, PD, KM, VS. Methodology: PD, RG. Project administration: TA, KM. Visualization: AK. Writing - original draft: RG. Writing - review and editing: VS, KM, PD, TA, AK.

Author ORCIDs

Romain Gastineau  <https://orcid.org/0000-0001-8661-5118>

Przemysław Dąbek  <https://orcid.org/0000-0002-3736-3011>

Kamila Mianowicz  <https://orcid.org/0000-0002-7755-3258>

Tomasz Abramowski  <https://orcid.org/0000-0002-9029-406X>

Data availability

All of the data that support the findings of this study are available in the main text.

References

- Amon DJ, Ziegler AF, Dahlgren TG, Glover AG, Goineau A, Gooday AJ, Wiklund H, Smith CR (2016) Insights into the abundance and diversity of abyssal megafauna in a polymetallic-nodule region in the eastern Clarion-Clipperton Zone. *Scientific Reports* 6(1): 30492. <https://doi.org/10.1038/srep30492>
- Amon DJ, Gollner S, Morato T, Smith CR, Chen C, Christiansen S, Currie B, Drazen JC, Fukushima T, Gianni M, Gjerde KM, Gooday AJ, Grillo GG, Haeckel M, Joyini T, Ju S-J, Levin LA, Metaxas A, Mianowicz K, Molodtsova TN, Narberhaus I, Orcutt BN, Swadling A, Tuhumwire J, Palacio PU, Walker M, Weaver P, Xu X-W, Mulalap CY, Edwards PE, Pickens C (2022) Assessment of scientific gaps related to the effective environmental management of deep-seabed mining. *Marine Policy* 138: 105006. <https://doi.org/10.1016/j.marpol.2022.105006>
- Baláz P (2022) Results of the second phase of deep-sea polymetallic nodules geological survey in Interoceanmetal Joint Organization licence area (2016–2021). *Mineralia Slovaca* 54(2): 95–118. <https://doi.org/10.56623/ms.2022.54.2.1>
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19(5): 455–477. <https://doi.org/10.1089/cmb.2012.0021>
- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf M, Stadler PF (2013) MITOS: Improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution* 69(2): 313–319. <https://doi.org/10.1016/j.ympev.2012.08.023>
- Bonifácio P, Neal L, Menot L (2021) Diversity of deep-sea scale-worms (Annelida, Polynoidae) in the Clarion-Clipperton Fracture Zone. *Frontiers in Marine Science* 8: 656899. <https://doi.org/10.3389/fmars.2021.656899>
- Bribiesca-Contreras G, Dahlgren TG, Horton T, Drazen JC, Drennan R, Jones DOB, Leitner AB, McQuaid KA, Smith CR, Taboada S, Wiklund H, Glover AG (2021) Biogeography and connectivity across habitat types and geographical scales in Pacific abyssal scavenging amphipods. *Frontiers in Marine Science* 8: 705237. <https://doi.org/10.3389/fmars.2021.705237>
- Bribiesca-Contreras G, Dahlgren TG, Amon DJ, Cairns S, Drennan R, Durden JM, Eléaume MP, Hosie AM, Kremenetskaia A, McQuaid K, O'Hara TD, Rabone M, Simon-Lledó E, Smith CR, Watling L, Wiklund H, Glover AG (2022) Benthic megafauna of the western Clarion-Clipperton Zone, Pacific Ocean. *ZooKeys* 1113: 1–110. <https://doi.org/10.3897/zookeys.1113.82172>
- Cairns SD (2016) New abyssal Primnoidae (Anthozoa: Octocorallia) from the Clarion-Clipperton Fracture Zone, equatorial Northeastern Pacific. *Marine Biodiversity* 46(1): 141–150. <https://doi.org/10.1007/s12526-015-0340-x>
- Cairns SD, Bayer FM (2008) A review of the Octocorallia (Cnidaria: Anthozoa) from Hawai'i and adjacent seamounts: the genus *Narella*. *Pacific Science* 62(1): 83–115. [https://doi.org/10.2984/1534-6188\(2008\)62\[83:AROTOC\]2.0.CO;2](https://doi.org/10.2984/1534-6188(2008)62[83:AROTOC]2.0.CO;2)
- Cairns SD, Wirshing HH (2018) A phylogenetic analysis of the Primnoidae (Anthozoa: Octocorallia: Calcaxonia) with analyses of character evolution and a key to the

- genera and subgenera. *BMC Evolutionary Biology* 18(1): 66. <https://doi.org/10.1186/s12862-018-1182-5>
- Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: Architecture and applications. *BMC Bioinformatics* 10(1): 421. <https://doi.org/10.1186/1471-2105-10-421>
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25(15): 1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>
- Choi EH, Shin C, Baek SY, Hwang J, Jun J, Jang KH, Ryu SH, Hwang UW (2020) The complete mitochondrial genome of *Plumarella spinosa* (Octocorallia: Calcaxonia: Primnoidae) from South Korea. *Mitochondrial DNA, Part B, Resources* 5(1): 725–726. <https://doi.org/10.1080/23802359.2020.1715275>
- Cordes EE, Jones DOB, Schlacher TA, Amon DJ, Bernardino AF, Brooke S, Carney R, DeLeo DM, Dunlop KM, Escobar-Briones EG, Gates AR, Génio L, Gobin J, Henry L-A, Herrera S, Hoyt S, Joye M, Kark S, Mestre NC, Metaxas A, Pfeifer S, Sink K, Sweetman AK, Witte U (2016) Environmental impacts of the deep-water oil and gas industry: A review to guide management strategies. *Frontiers in Environmental Science* 4: 58. <https://doi.org/10.3389/fenvs.2016.00058>
- Dahlgren TG, Wiklund H, Rabone M, Amon DJ, Ikebe C, Watling L, Smith CR, Glover AG (2016) Abyssal fauna of the UK-1 polymetallic nodule exploration area, Clarion-Clipperton Zone, central Pacific Ocean: Cnidaria. *Biodiversity Data Journal* 4: e9277. <https://doi.org/10.3897/BDJ.4.e9277>
- Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* 37(1): 291–294. <https://doi.org/10.1093/molbev/msz189>
- Durden JM, Putts M, Bingo S, Leitner AB, Drazen JC, Gooday AJ, Jones DOB, Sweetman AK, Washburn TW, Smith CR (2021) Megafaunal ecology of the western Clarion Clipperton Zone. *Frontiers in Marine Science* 8: 671062. <https://doi.org/10.3389/fmars.2021.671062>
- Figueroa DF, Baco AR (2015) Octocoral mitochondrial genomes provide insights into the phylogenetic history of gene order rearrangements, order reversals, and cnidarian phylogenetics. *Genome Biology and Evolution* 7(1): 391–409. <https://doi.org/10.1093/gbe/evu286>
- Goineau A, Gooday AJ (2019) Diversity and spatial patterns of foraminiferal assemblages in the eastern Clarion–Clipperton zone (abyssal eastern equatorial Pacific). *Deep-sea Research, Part I, Oceanographic Research Papers* 149: 103036. <https://doi.org/10.1016/j.dsr.2019.04.014>
- Gooday AJ, Wawrzyniak-Wydrowska B (2023) Macrofauna-sized foraminifera in epibenthic sledge samples from five areas in the eastern Clarion-Clipperton Zone (equatorial Pacific). *Frontiers in Marine Science* 9: 1059616. <https://doi.org/10.3389/fmars.2022.1059616>
- Gooday AJ, Holzmann M, Goineau A, Kamenskaya O, Melnik VF, Pearce RB, Weber AA, Pawlowski J (2018) Xenophyophores (Rhizaria, Foraminifera) from the eastern Clarion-Clipperton Zone (equatorial Pacific): The genus *Psammmina*. *Protist* 169(6): 926–957. <https://doi.org/10.1016/j.protis.2018.09.003>
- Gooday AJ, Durden JM, Holzmann M, Pawlowski J, Smith CR (2020) Xenophyophores (Rhizaria, Foraminifera), including four new species and two new genera, from the western Clarion-Clipperton Zone (abyssal equatorial Pacific). *European Journal of Protistology* 75: 125715. <https://doi.org/10.1016/j.ejop.2020.125715>

- Gooday AJ, Lejzerowicz F, Goineau A, Holzmänn M, Kamenskaya O, Kitazato H, Lim S-C, Pawlowski J, Radziejewska T, Stachowska Z, Wawrzyniak-Wydrowska B (2021) The biodiversity and distribution of abyssal benthic foraminifera and their possible ecological roles: A synthesis across the Clarion-Clipperton Zone. *Frontiers in Marine Science* 8: 634726. <https://doi.org/10.3389/fmars.2021.634726>
- Hellberg ME (2006) No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology* 6(1): 24. <https://doi.org/10.1186/1471-2148-6-24>
- Hickson SJ (1904) The Alcyonaria of the Cape of Good Hope, part II. *Marine Investigations in South Africa* 3: 211–239. [pls 7–9] <https://doi.org/10.5962/p.366544>
- Hikov A, Milakovska Z, Stoyanova V, Stefanova E, Abramowski T, Chavdarova S, Stavrev M (2022) REE and trace elements distribution in the deep-sea sediments from the Interoceanmetal (IOM) Polymetallic Nodule Exploration Area in the Clarion-Clipperton Fractures Zone, NE Pacific. *Proceedings of the Bulgarian Academy of Sciences* 75(7): 1018–1027. <https://doi.org/10.7546/crabs.2022.07.10>
- ISA (2010) A geological model of polymetallic nodule deposits in the Clarion-Clipperton Fracture Zone and prospector's guide for polymetallic nodule deposits in the Clarion Clipperton Fracture Zone. Technical Study: No. 6. International Seabed Authority, Kingston, Jamaica. <https://www.isa.org.jm/publications/technical-study-6-a-geological-model-of-polymetallic-nodule-deposits-in-the-clarion-clipperton-fracture-zone/> [Accessed on: 2023-10-13]
- ISA (2015) Consolidated Regulations and Recommendations on Prospecting and Exploration. Revised edition. International Seabed Authority, Kingston, Jamaica. <https://www.isa.org.jm/publications/consolidated-regulations-and-recommendations-on-prospecting-and-exploration/> [Accessed on: 2023-10-13]
- ISBA/21/LTC/15/Corr.1 (2021) Recommendations for the guidance of contractors on the content, format and structure of annual reports. International Seabed Authority, Kingston, Jamaica. <https://www.isa.org.jm/documents/isba-21-ltc-15-corr-1/> [Accessed on: 2023-10-13]
- ISBA/25/LTC/6/Rev.2 (2022) Recommendations for the guidance of contractors on the content, format and structure of annual reports. International Seabed Authority, Kingston, Jamaica. https://www.isa.org.jm/wp-content/uploads/2022/04/ISBA_25_LTC_6_Rev.2-2211076E.pdf [Accessed on: 2023-10-13]
- Janssen A, Stuckas H, Vink A, Arbizu PM (2019) Biogeography and population structure of predominant macrofaunal taxa (Annelida and Isopoda) in abyssal polymetallic nodule fields: Implications for conservation and management. *Marine Biodiversity* 49(6): 2641–2658. <https://doi.org/10.1007/s12526-019-00997-1>
- Johnson MG, Gardner EM, Liu Y, Medina R, Goffinet B, Shaw AJ, Zerega NJ, Wickett NJ (2016) HybPiper: extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. *Applications in Plant Sciences* 4(7): apps.1600016. <https://doi.org/10.3732/apps.1600016>
- Jones DOB, Simon-Lledó E, Amon DJ, Bett BJ, Caulle C, Clément L, Connelly DP, Dahlgren TG, Durden JM, Drazen JC, Felden J, Gates AR, Georgieva MN, Glover AG, Gooday AJ, Hollingsworth AL, Horton T, James RH, Jeffreys RM, Laguionie-Marchais C, Leitner AB, Lichtschlag A, Menendez A, Paterson GLJ, Peel K, Robert K, Schoening T, Shulga NA, Smith CR, Taboada S, Thurnherr AM, Wiklund H, Young CR, Huvenne VAI (2021) Environment, ecology, and potential effectiveness of an area protected from deep-sea mining (Clarion Clipperton Zone, abyssal Pacific). *Progress in Oceanography* 197: 102653. <https://doi.org/10.1016/j.pocean.2021.102653>

- Kamenskaya OE, Gooday AJ, Radziejewska T, Wawrzyniak-Wydrowska B (2012) Large, enigmatic foraminiferan-like protists in the eastern part of the Clarion-Clipperton Fracture Zone (abyssal eastern equatorial Pacific): Biodiversity and vertical distribution in the sediment. *Marine Biodiversity* 42(3): 311–327. <https://doi.org/10.1007/s12526-012-0114-7>
- Kamenskaya OE, Gooday AJ, Tendal OS, Melnik VP (2015) Xenophyophores (Protista, Foraminifera) from the Clarion-Clipperton fracture zone with description of three new species. *Marine Biodiversity* 45(3): 581–593. <https://doi.org/10.1007/s12526-015-0330-z>
- Kamenskaya OE, Gooday AJ, Tendal OS, Melnik VF (2017) Xenophyophores (Rhizaria, Foraminifera) from the Russian license area of the Clarion-Clipperton Zone (eastern equatorial Pacific), with the description of three new species. *Marine Biodiversity* 47(2): 299–306. <https://doi.org/10.1007/s12526-016-0595-x>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kersten O, Vetter EW, Jungbluth MJ, Smith CR, Goetze E (2019) Larval assemblages over the abyssal plain in the Pacific are highly diverse and spatially patchy. *PeerJ* 7: e7691. <https://doi.org/10.7717/peerj.7691>
- Kinoshita K (1907) Vorläufige Mitteilung über einige neue japanische Primnoid-Korallen. *Annotationes Zoologicae Japonenses* 6(3): 229–237.
- Kuhn T, Rühlemann C (2021) Exploration of polymetallic nodules and resource assessment: A case study from the German Contract Area in the Clarion-Clipperton Zone of the tropical Northeast Pacific. *Minerals (Basel)* 11(6): 618. <https://doi.org/10.3390/min11060618>
- Laming SR, Christodoulou M, Martinez Arbizu P, Hilário A (2021) Comparative reproductive biology of deep-sea ophiuroids inhabiting polymetallic-nodule fields in the Clarion-Clipperton Fracture Zone. *Frontiers in Marine Science* 8: 663798. <https://doi.org/10.3389/fmars.2021.663798>
- Lejzerowicz F, Gooday AJ, Barrenechea Angeles I, Cordier T, Morard R, Apothéloz-Perret-Gentil L, Lins L, Menot L, Brandt A, Levin LA, Martinez Arbizu P, Smith CR, Pawłowski J (2021) Eukaryotic biodiversity and spatial patterns in the Clarion-Clipperton Zone and other abyssal regions: Insights from sediment DNA and RNA metabarcoding. *Frontiers in Marine Science* 8: 671033. <https://doi.org/10.3389/fmars.2021.671033>
- Lim S, Wiklund H, Glover AG, Dahlgren TG, Tan KS (2017) A new genus and species of abyssal sponge commonly encrusting polymetallic nodules in the Clarion-Clipperton Zone, East Pacific Ocean. *Systematics and Biodiversity* 15(6): 507–519. <https://doi.org/10.1080/14772000.2017.1358218>
- Lohse M, Drechsel O, Kahlau S, Bock R (2013) OrganellarGenomeDRAW – A suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. *Nucleic Acids Research* 41(W1): W575–W581. <https://doi.org/10.1093/nar/gkt289>
- Milakovska Z, Hikov A, Stoyanova V, Peytcheva I, Lyubomirova V, Abramowski T (2022) REY in pore waters of sediments hosting Fe-Mn nodules of the Interoceanmetal exploration area in the Clarion-Clipperton Fracture Zone, NE Pacific. *Geologica Balcanica* 51(2): 27–35. <https://doi.org/10.52321/GeolBalc.51.2.27>
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37(5): 1530–1534. <https://doi.org/10.1093/molbev/msaa015>

- Muthye V, Lavrov DV (2021) Multiple losses of MSH1, gain of mtMutS, and other changes in the MutS family of DNA repair proteins in animals. *Genome Biology and Evolution* 13(9): evab191. <https://doi.org/10.1093/gbe/evab191>
- Muthye V, Mackereth CD, Stewart JB, Lavrov DV (2022) Large dataset of octocoral mitochondrial genomes provides new insights into mt-mutS evolution and function. *DNA Repair* 110: 103273. <https://doi.org/10.1016/j.dnarep.2022.103273>
- Neal L, Wiklund H, Gunton LM, Rabone M, Bribiesca-Contreras G, Dahlgren TG, Glover AG (2022) Abyssal fauna of polymetallic nodule exploration areas, eastern Clarion-Clipperton Zone, central Pacific Ocean: Amphinomidae and Euphrosinidae (Annelida, Amphinomida). *ZooKeys* 1137: 33–74. <https://doi.org/10.3897/zookeys.1137.86150>
- Patel T, Robert H, D'Udekem D'Acoz C, Martens K, De Mesel I, Degraer S, Schön I (2020) Biogeography and community structure of abyssal scavenging Amphipoda (Crustacea) in the Pacific Ocean. *Biogeosciences* 17(10): 2731–2744. <https://doi.org/10.5194/bg-17-2731-2020>
- Quek RZB, Jain SS, Neo ML, Rouse GW, Huang D (2020) Transcriptome-based target-enrichment baits for stony corals (Cnidaria: Anthozoa: Scleractinia). *Molecular Ecology Resources* 20(3): 807–818. <https://doi.org/10.1111/1755-0998.13150>
- Quek ZBR, Jain SS, Richards ZT, Arrigoni R, Benzoni F, Hoeksema BW, Carvajal JI, Wilson NG, Baird AH, Kitahara MV, Seiblitiz IGL, Vaga CF, Huang D (2023) A hybrid-capture approach to reconstruct the phylogeny of Scleractinia (Cnidaria: Hexacorallia). *Molecular Phylogenetics and Evolution* 186: 107867. <https://doi.org/10.1016/j.ympev.2023.107867>
- Rabone M, Wiethase JH, Simon-Lledó E, Emery AM, Jones DOB, Dahlgren TG, Bribiesca-Contreras G, Wiklund H, Horton T, Glover AG (2023) How many metazoan species live in the world's largest mineral exploration region? *Current biology* 33(12): 2383–2396.e5. <https://doi.org/10.1016/j.cub.2023.04.052>
- Ramos NI, DeLeo DM, Horowitz J, McFadden CS, Quattrini AM (2023) Selection in coral mitogenomes, with insights into adaptations in the deep sea. *Scientific Reports* 13(1): 6016. <https://doi.org/10.1038/s41598-023-31243-1>
- Riehl T, De Smet B (2020) *Macrostylis metallica* spec. nov.—An isopod with geographically clustered genetic variability from a polymetallic-nodule area in the Clarion-Clipperton Fracture Zone. *PeerJ* 8: e8621. <https://doi.org/10.7717/peerj.8621>
- Roark EB, Guilderson TP, Dunbar RB, Fallon SJ, Mucciarone DA (2009) Extreme longevity in proteinaceous deep-sea corals. *Proceedings of the National Academy of Sciences of the United States of America* 106(13): 5204–5208. <https://doi.org/10.1073/pnas.0810875106>
- Rodd C, Whalan S, Humphrey C, Harrison PL (2022) Enhancing coral settlement through a novel larval feeding protocol. *Frontiers in Marine Sciences* 9:918232. <https://doi.org/10.3389/fmars.2022.918232>
- Shearer TL, Van Oppen MJ, Romano SL, Wörheide G (2002) Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology* 11(12): 2475–2487. <https://doi.org/10.1046/j.1365-294X.2002.01652.x>
- Simon-Lledó E, Pomee C, Ahokava A, Drazen JC, Leitner A, Flynn AJ, Parianos J, Jones DO (2020) Multi-scale variations in invertebrate and fish megafauna in the mid-eastern Clarion Clipperton Zone. *Progress in Oceanography* 2020 187: 102405. <https://doi.org/10.1016/j.pocean.2020.102405>
- Skowronek A, Maciąg Ł, Zawadzki D, Strzelecka A, Baláž P, Mianowicz K, Abramowski T, Konečný P, Krawcewicz A (2021) Chemostratigraphic and textural indicators of nucleation and growth of polymetallic nodules from the Clarion-Clipperton Fracture Zone (IOM Claim Area). *Minerals (Basel)* 11(8): 868. <https://doi.org/10.3390/min11080868>

- Smith SA, Dunn CW (2008) Phyutility: A phyloinformatics tool for trees, alignments and molecular data. *Bioinformatics* 24(5): 715–716. <https://doi.org/10.1093/bioinformatics/btm619>
- Smith PJ, McVeagh SM, Mingoia JT, France SC (2004) Mitochondrial DNA sequence variation in deep-sea bamboo coral (Keratoisidinae) species in the southwest and northwest Pacific Ocean. *Marine Biology* 144(2): 253–261. <https://doi.org/10.1007/s00227-003-1206-5>
- Stachowska-Kamińska Z, Gooday AJ, Radziejewska T, Arbizu PM (2022) Macrofaunal foraminifera from a former benthic impact experiment site (IOM contract area) in the abyssal eastern Clarion-Clipperton Zone. *Deep-sea Research, Part I, Oceanographic Research Papers* 188: 103848. <https://doi.org/10.1016/j.dsr.2022.103848>
- Stewart ECD, Bribiesca-Contreras G, Taboada S, Wiklund H, Ravara A, Pape E, De Smet B, Neal L, Cunha MR, Jones DOB, Smith CR, Glover AG, Dahlgren TG (2023) Biodiversity, biogeography, and connectivity of polychaetes in the world’s largest marine minerals exploration frontier. *Diversity & Distributions* 29(6): 727–747. <https://doi.org/10.1111/ddi.13690>
- Štyriaková D, Štyriaková I, Šuba J, Baláž P, Abramowski T (2022) Bioleaching test of polymetallic nodule samples from the IOM Exploration Area. *Minerals (Basel)* 12(11): 1373. <https://doi.org/10.3390/min12111373>
- Taboada S, Kenny NJ, Riesgo A, Wiklund H, Paterson GLJ, Dahlgren TG, Glover AG (2018a) Mitochondrial genome and polymorphic microsatellite markers from the abyssal sponge *Plenaster craigi* Lim & Wiklund, 2017: Tools for understanding the impact of deep-sea mining. *Marine Biodiversity* 48(1): 621–630. <https://doi.org/10.1007/s12526-017-0786-0>
- Taboada S, Riesgo A, Wiklund H, Paterson GLJ, Koutsouveli V, Santodomingo N, Dale AC, Smith CR, Jones DOB, Dahlgren TG, Glover AG (2018b) Implications of population connectivity studies for the design of marine protected areas in the deep sea: An example of a demosponge from the Clarion-Clipperton Zone. *Molecular Ecology* 27(23): 4657–4679. <https://doi.org/10.1111/mec.14888>
- Uhlenkott K, Meyn K, Vink A, Arbizu PM (2023) A review of megafauna diversity and abundance in an exploration area for polymetallic nodules in the eastern part of the Clarion Clipperton Fracture Zone (North East Pacific), and implications for potential future deep-sea mining in this area. *Marine Biodiversity* 53(2): 22. <https://doi.org/10.1007/s12526-022-01326-9>
- Van Nijen K, Van Passel S, Squires D (2018) A stochastic techno-economic assessment of seabed mining of polymetallic nodules in the Clarion Clipperton Fracture Zone. *Marine Policy* 95: 133–141. <https://doi.org/10.1016/j.marpol.2018.02.027>
- Verrill AE (1864) List of the polyps and corals sent by the Museum of Comparative Zoology to other institutions in exchange, with annotations. *Bulletin of the Museum of Comparative Zoology* 1: 29–60.
- Watanabe T, Nishida M, Watanabe K, Wewengkang DS, Hidaka M (2005) Polymorphism in nucleotide sequence of mitochondrial intergenic region in scleractinian coral (*Galaxea fascicularis*). *Marine Biotechnology (New York, N.Y.)* 7(1): 33–39. <https://doi.org/10.1007/s10126-004-3200-4>
- Weaver PPE, Aguzzi J, Boschen-Rose RE, Colaço A, de Stigter H, Gollner S, Haeckel M, Hauton C, Helmons R, Jones DOB, Lily H, Mestre NC, Mohn C, Thomsen L (2022) Assessing plume impacts caused by polymetallic nodule mining vehicles. *Marine Policy* 139: 105011. <https://doi.org/10.1016/j.marpol.2022.105011>